EFFECT OF UNCOUPLERS AND ADRY REAGENTS ON DELAYED AND TRIGGERED EMISSION FROM ISOLATED CHLOROPLASTS*

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Received 26 July 1976

1. Introduction

The effect of uncouplers on msec delayed luminescence from chloroplasts is well documented [1–6] and is related to the abolishment by uncouplers of transmembrane potential and pH gradients which enhance delayed luminescence considerably [3,6,7]. The situation with respect to delayed luminescence in longer time region (> 100 msec) and triggered luminescence is much less certain. Mayne and Clayton reported that acid-base triggered luminescence was inhibited by uncouplers [8]. In our lab we noticed previously [9] that in the case of solvent triggered luminescence the only uncoupler that inhibited this luminescence was FCCP (or CCCP).

Several authors noticed the inhibitory effect of some uncouplers on delayed and triggered light.

It is known that some uncouplers affect also the precursors of oxygen evolution, besides their effect as uncouplers of phosphorylation. These reagents were termed Accelerating of the Deactivation of the Reaction of the water splitting enzyme Y (ADRY) reagents. Their effect was shown by the decrease of the yield of oxygen evolution excited by flashes at longer time intervals between the flashes [10]; more specifically they accelerate the deactivation of higher S-states [11].

* *Abbreviations:* ADRY reagents, reagents acceleration of the deactivation of the reaction of the water-splitting enzyme Y; FCCP, carbonylcyanide-P-trifluoromethoxy phenylhydrazone; ANT 2a, 2-(4 chloro)anilino-3,5-dintrothiophen; ANT 2f, 2-(4-dimethylamine)anilino-3,5dinitrothiophen; ANT 2p, 2-(3 chloro-4 trifluoromethyl)anilino-3,5 dinitrothiophen.

Since luminescence was shown to depend in a strong way on the S-state [12–15] and presumably is generated from a recombination involving the oxygen precursors, it could be possible that the effect of uncouplers, in some cases at least, is not due to their uncoupling effect, but to ADRY effect causing a depletion in the luminescence precursors.

In this work an attempt was made to differentiate between these two groups with respect to their effect on luminescence, with the result that indeed only uncouplers that show ADRY effect abolish all kinds of triggered luminescence and delayed light in the second time region. The other uncouplers do not inhibit and sometimes even enhance the luminescence.

2. Materials and methods

Chloroplasts were prepared as described by Avron [16] and stored at liquid nitrogen. The chloroplasts were able to phosphorylate after thawing, and the phosphorylation stopped upon addition of gramicidin, accompanied with increase in oxygen evolution. These results ensured us that the frozen chloroplasts were coupled. The luminescence measurements were carried out by an instrument previously described [17]. The delayed light emitted by the chloroplasts was measured from about 100 msec after the end of the preillumination. The different types of induced luminescence were triggered as described before [17]. The light source was a continuous lamp (slide projector) filtered by $CuSO_4$ solution and a cut-off glass filter ($\lambda > 530$ nm), which transmitted a band between 530 and 600 nm. ADRY reagents used besides FCCP were kindly sent to us as a gift by Dr G. Renger.

3. Results and discussion

Recorded traces of typical luminescence events are shown in figs.1–5. These recordings show that all kinds of triggered luminescence tested (acid, acid-base, salt, T-jump, solvent) as well as delayed luminescence at time > 100 msec were affected in the same manner: they were severely inhibited by the ADRY reagent (ANT 2p) but only little affected by gramicidin or NH_4Cl.

This phenomenon was repeated with all the ADRY

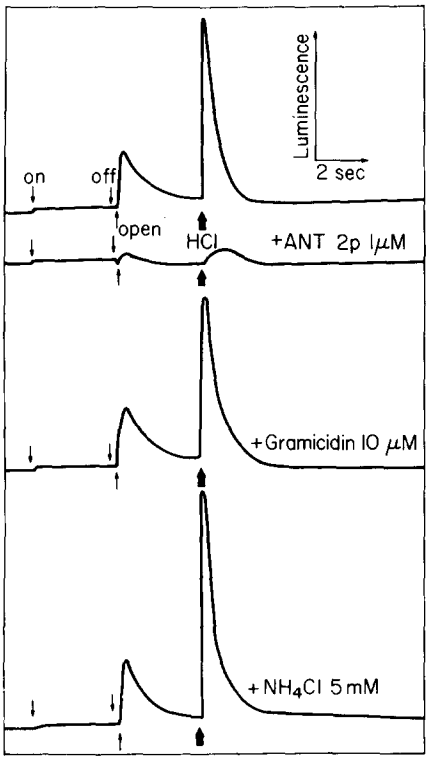

Fig.1. Acid induced luminescence – demonstration of the effects of uncouplers and ADRY reagents. The curves represent luminescence as a function of time. The reaction mixture contained: 0.1 M sucrose, 50 mM NaCl, 1 mM $MgCl_2$ and 25 mM Tris-buffer pH 7.8. Chloroplast concentration: 30 μg chlorophyl/ml, in a total volume of 2 ml. In addition, 1 μM ANT 2p, 10 μM gramicidin or 5 mM NH_4Cl were added where mentioned. 'On' and 'off' arrows represent exciting light on and off, respectively. 'Open' corresponds to opening of the shutter between the sample and a photomultiplier ~100 msec after the end of illumination. The heavy arrow represents injection of HCl to the chloroplasts suspension.

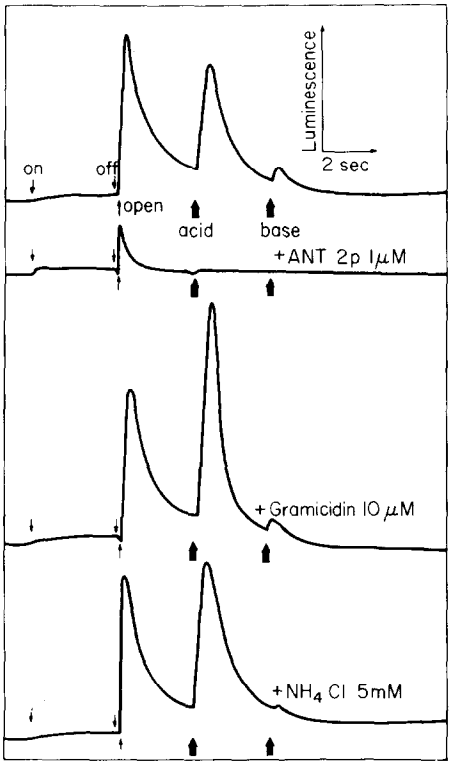

Fig.2

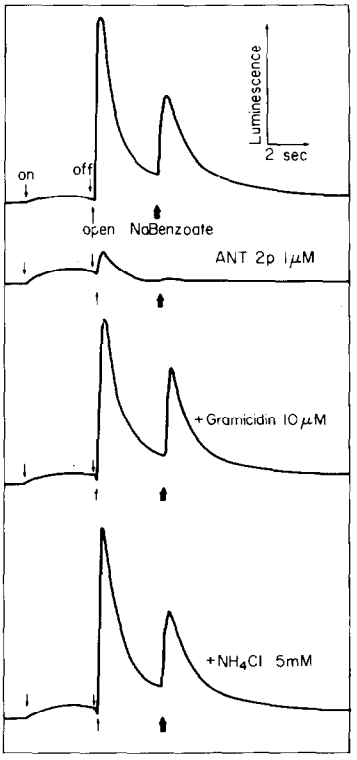

Fig.3

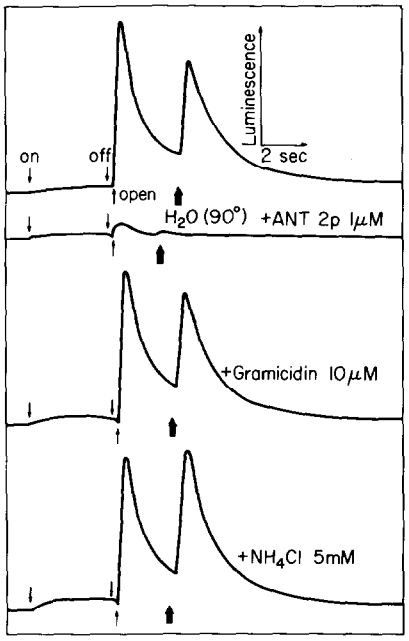

Fig.4. T-jump induced luminescence – demonstration of the effect of uncouplers and ADRY reagents. Conditions are the same as in fig.1. Triggering luminescence by injection of 1 ml of water in 90°C.

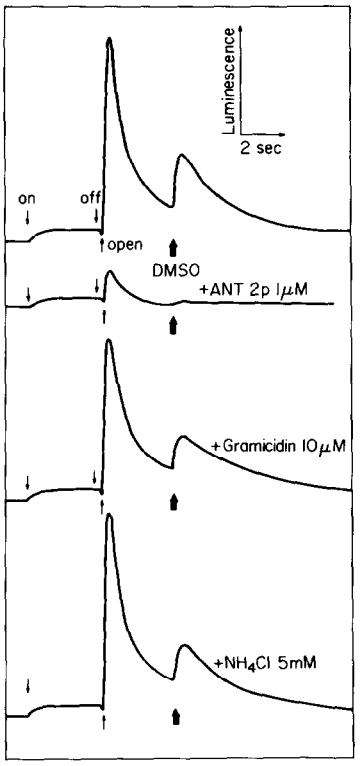

Fig.5. Solvent induced luminescence – demonstration of the effect of uncouplers and ADRY reagents. The same conditions as in fig.1. Triggering luminescence by injection of dimethylsulfoxide (DMSO).

reagents available to us (ANT 2a, ANT 2p, ANT 2f and FCCP). The ability of the various ADRY reagents to inhibit luminescence was not the same; this is expressed by the requirement of different concentrations for equal inhibition degrees. Some samples of concentration–inhibition curves are shown in fig.6. From such studies the general inhibition ability is in the order ANT 2f < ANT 2a < FCCP < ANT 2p. This order is the same for their effect on the decay of oxygen evolution precursors [10,11].

Fig.2. Acid-base induced luminescences – demonstration of the effect of uncouplers and ADRY reagents. The same conditions as in fig.1, except that two triggering events following one another are demonstrated, 'acid' corresponds to injection of 0.5 ml succinic acid (0.1 M, pH 4.3) and 'base' injection of 0.5 ml Tris (0.5 M, pH 8.3).

Fig.3. Salt induced luminescence – demonstration of the effect of uncouplers and ADRY reagents. The same conditions as in fig.1, except that 1.75 M sodium benzoate (Na benzoate) replaces HCl.

Uncouplers which are not ADRY reagents, like gramicidin, nigericin, NH_4Cl did not inhibit luminescence in a concentration range where uncoupling occurs. Delayed luminescence was not affected at all. The triggered luminescences were modulated in an uncontrolled way but never attenuated: in several experiments we obtained large enhancement of HCl induced luminescence with addition of gramicidin. Sometimes, however, this enhancement did not occur. Also a large enhancement of T-jump luminescence was obtained with the addition of nigericin. NH_4Cl modulated luminescence to relatively small extent (fig.7). The difference in the behaviour of ADRY reagents and uncouplers is very evident, comparing figs.6 and 7.

The lack of effect of uncoupling on luminescence in our case, compared to the effect on msec delayed

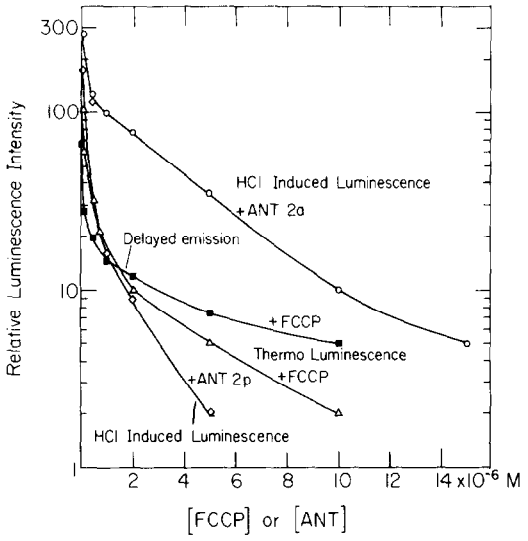

Fig.6. Effect of ADRY reagents on delayed emission and triggered luminescence. Maximal luminescence intensity is plotted vs. different concentration of FCCP, ANT 2a or ANT 2p.

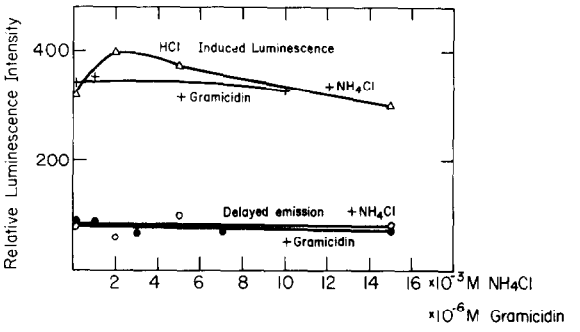

Fig.7. Effect of gramicidin and NH_4Cl on delayed emission and HCl-induced luminescence, the maximum of the luminescence signal as shown in fig.1 is plotted vs. different concentration of uncouplers which were present in the reaction mixture prior to illumination.

luminescence may be explained simply by the small degree of membrane gradients obtained under our conditions: (a) there is very limited steady-state electron transport (no electron acceptors were added); (b) during the time between preillumination and delayed luminescence (or triggered luminescence) the membrane gradients decay. Thus the luminescence obtained in our conditions is already equivalent to an 'uncoupled' state.

The specific effect of ADRY reagents supply an additional evidence that oxygen precursors are involved in the luminescence process.

It must be noted that no distinction was made in the past between the effect of 'pure' uncouplers and ADRY reagents on luminescence. Very often FCCP was used to demonstrate an uncoupling effect. The distinction between the two kinds of reagents is significant.

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